SPECIES

Geometric morphometric shape and size analysis of endemic black Clam, *Villorita cyprinoides* (Gray, 1825) (Mollusca: Bivalvia: Cyrenidae) from Koottayi estuary, Kerala, South India

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ABSTRACT

Geometric morphometric allowed us to study the shape, size and asymmetry of the right and left shells of the highly economically important endemic black clam *Villorita cyprinoides* (Gray, 1825) local population representing the Koottayi estuary, Tirur-Ponnani River, Kerala, South India. As far as we know, nobody has studied the population structure of black clams from southern India and this is the first geometric morphometric analysis of endemic black clam, which provided preliminary information on the population structure of black clam from the Koottayi estuary. Our study area faces a high level of pollution and anthropogenic intervention; apart from that, opens near the Arabian Sea's Barmouth. Using various multivariate statistical methods, we concluded that right and left shell halves are not of the same size and shape; and asymmetry exists. The high level of pollution and anthropogenic effect may be the reason for the existence of fluctuation asymmetry. Our research will serve as baseline information to evaluate the effects of pollution or environmental stress and human interference on endemic species. Evaluation of morphological variations works as a critical tool for future studies such as developmental instability, natural selection, phenotypic adaptation and evolution.

Keywords: Black clam, endemic species, estuary, geometric morphometrics, phenotypic plasticity, shell shape and size

1. INTRODUCTION

The fact that phenotypic variations are a reflection of adaptation or phenotypic plasticity or both. It is very challenging to explain the vital environmental selection pressure mechanisms behind phenotypic variations and how they work. Variations do not inherently inhibit adaptation due to phenotypic plasticity and promote genetic divergence in some conditions (Thimbert-Plante and Hendry, 2011; Fitzpatrick, 2012; Dowle et al., 2015). Phenotypic variations stabilize the

successful colonization of the individuals in a marginal environment and subsequently, contribute to genetic differentiation (Fitzpatrick, 2012). The interaction between the phenotype and the environment will contribute to the emergence of new distinct local phenotypic variations (Dowle et al., 2015; Fassatoui et al., 2019). Habitat-specific convergent evolution and phenotypic variations would be used to test adaptive hypotheses (Minards et al., 2014).

Some shelled gastropod species showed phenotypic plastic responses related to the environmental and predator pressure (Appleton and Palmer, 1988; Trussell, 2000; Doyle et al., 2010; Hollander and Butlin, 2010; Butlin et al., 2014). However, the shell characteristics can also represent a fixed genetic character (Goodfriend, 1986; Johannesson, 1996; Stankowski, 2013). Compared to linear measurements, geometric morphometrics (GMM) are now commonly used for studying the population structure of organism, particularly in inter-and intra-specific variations. Looking at the asymmetry of species is one of the fundamental aspects of GMM, which will provide valuable information on the developmental basis of morphological integration and phenotypic plasticity (Lajus et al., 2015). Mainly three different forms of asymmetry are present, such as directional asymmetry (DA), fluctuating asymmetry (FA) and antisymmetry (AS); compared to three asymmetries, FA does use as a biomarker for environmental stress assessment. Increased levels of FA seem related to various sources of stress, such as pollution (Franco et al., 2002), parasitism (Escos et al., 1995), stressful El Nino conditions (Alados et al., 1993) and poor feeding conditions (Somarakis et al., 1997). The variation in FA has already been related to a reduction in fitness performance and other population characteristics such as growth, morphology, survival, reproduction and longevity (Allenbach et al., 1999; Borrell et al., 2004; Kristoffersen and Magoulas, 2008; Arambourou et al., 2012).

Villorita cyprinoides (Gray, 1825) is an economically important endemic black clam found along the Indian coast (Jones, 1968). The black clams provide a rich and inexpensive protein source for local consumption and export purposes. Besides, shells have an immense commercial value, as they are raw materials for the manufacture of cement, calcium carbide, sand-lime bricks, distemper, glass, rayon, paper, sugar, pharmaceuticals, pesticide and poultry feed. The raw shells used in paddy fields and fish farms for neutralizing acid soil, add as slaked lime (Sudha, 1991; Joe, 1993; Kripa and Joseph, 1993; Krishna and Ammini, 2017). Clam habitat also offers many valuable ecological services; clam deposits avoid large-scale soil erosion and stabilize nearby islands (Kripa and Joseph, 1993; Krishna and Ammini, 2017). The population structure of *V. cyprinoides* is well known from the Vembanad lake, Kerala, however, the basic population structure of black clam from the other areas of southern India is lacking (Suja and Mohammed, 2012; Krishna and Ammini, 2017). Vembanad Lake is a transitional ecotone between the sea and the inland lake ecosystem protected by a mud bund (largest in India). It effectively prevents saline intrusion from a highly productive environment that supports the feeding, spawning and rearing of a large number of commercial fish and shellfish (ATREE, 2007). The lake bed is unsuitable for the growth of *V. cyprinoides* due to industrial dredging for sub-fossil lime shells and the unregulated closure of the Thanneermukkom barrage (Pillai, 1991; Laxmilatha and Appukuttan, 2002). Closing the Thanneermukkom barrage prevents the salinity intrusion into the upstream lake area that was once the highest black clam density. And it also prevents the movement of clam larvae from downstream to the upstream region of the lake. Unregulated salinity gradients and the accumulation of toxic compounds in the lake's upstream area (stagnant water type – prevent the flushing out of accumulated substances) alter the black clam habitat (Pillai, 1991; Laxmilatha and Appukuttan, 2002; Krishna and Ammini, 2017). In 2015, black clam accounted for around 75% of India's total clam production and the clam harvest decreased by 29.72% (CMFRI, 2015).

As a preliminary investigation, we selected one of Kerala's important estuarine areas, Southern India, i.e., Koottayi estuary, Tirur-Ponnani River, which is subjected to ecological deterioration due to over increasing human interventions. Koottayi estuary is opposite to Ponnani estuary and tides from the Arabian Sea influence both the estuarine system and the water is brackish almost throughout the year. Tirur-Ponnani River is joined with the Bharathapuzha River, which flows into the Arabian Sea. The river course is noted for mangroves and is home to many species of fish and migratory and native birds (DTPC, 2020). Environmental stresses, such as a change in pH, salinity, pollution, anthropogenic impacts, etc., can cause a developmental deformity in the organism and lead to a high population asymmetry. Therefore, we hypothesized that studying the endemic species' population structure from this estuarine would explain environmental health conditions' preliminary characteristics. When higher levels of asymmetry were observed in the population, it insinuated that the presence of developmental noise did trigger by environmental stress. Asymmetry analysis is a morphological marker for environmental stress assessment (Scalici et al., 2017).

This study examined the morphological shape and size of the endemic black clam, *V. cyprinoides* right and left shells from Koottayi estuary, Tirur-Ponnani River, Kerala, south India. In shell asymmetry analysis, we distinguished the right and left shells of endemic black clam and the right-left shell asymmetry. Variations in shell shape are visualized with the aid of the principal component analysis (PCA). We also recorded the size and shape covariation between the right and left shells (using 2B-PLS) and the shells' developmental covariation (PLS1 analysis).

2. MATERIALS AND METHODS

Sample collection

The mature organism *Villorita cyprinoides* (Gray, 1825) was collected from the Koottayi estuary (10.83’87° N, 75.55’13° E), Tirur-Ponnani River, located in the Malappuram district of Kerala, India (Figure 1A, B, C). All samples were collected during the same period (March to May, summer season, 2017); the collection was carried out with clam fishermen’s help. The principal collection method is by diving underwater and collecting the clams by hand – collection estuary areas from 10 to 30 m in length and about 10 to 20 m in width and with a maximum water depth is 2.1 – 2.7 m. Specimens were transported alive to the laboratory and mechanically removed the flesh from the shell. The undamaged and clean shells were sorted and used for geometric morphometric analysis.

Figure 1 (A) Distribution map of *Villorita cyprinoides* – Data generated in OBIS server; (B) South India collection locality; (C) Collection site, Koottayi estuary

Geometric morphometric analysis

The collected shells were well cleaned and separated as left and right halves. A total of 101 right and 108 left ventral shell halves of *V. cyprinoides* were photographed using Canon EOS 7D (F-stop 22-27, Focal length – 180mm). Based on taxonomic characterization (Souji, 2018), 16 homologous landmarks were identified in the central region of both the left and right halves of the shell (Figure 2A, B, C) (Table 1). For land marking, 2D images of both halves of the shell were converted into tps file format using tps Utility V1.68 and tpsDig2 V2.26 used for land marking of the specimens (Rohlf, 2015). The x and y coordinates of these landmarks on the images were used to analyze asymmetry, allometry and covariations.

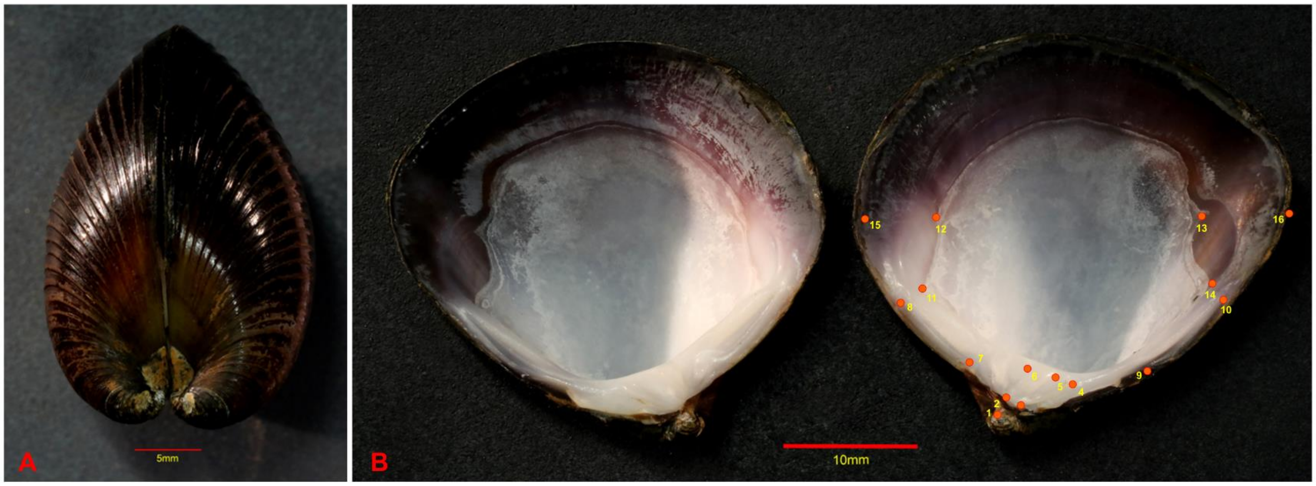

Figure 2 (A) Endemic black clam, *Villorita cyprinoides*; (B) Ventral region of both right and left halves of endemic black clam with selected geometric morphometric homologous landmarks used for shape and size analysis

Table 1 Geometric morphometric homologous landmarks used for shape and size analysis of *V. cyprinoides*

Landmark	Description
1	Umbo
2	Dorsal tip of Middle Cardinal Tooth
3	Dorsal tip of Posterior Cardinal Tooth
4	Antero-Ventral tip of Posterior Cardinal Tooth
5	Postero-Ventral tip of Middle Cardinal Tooth
6	Antero-Ventral tip of Middle Cardinal Tooth
7	Anterior end of Anterior Lateral Tooth
8	Posterior end of Anterior Lateral Tooth
9	Anterior end of Posterior Lateral Tooth
10	Posterior end of Posterior Lateral Tooth
11	Dorsal tip of Anterior Adductor Muscle
12	Ventral tip of Anterior Adductor Muscle
13	Ventral tip of Posterior Adductor Muscle
14	Dorsal tip of Posterior Adductor Muscle
15	Opposite end of Umbo
16	Opposite end of Ventral tip of Anterior Adductor Muscle
17	Opposite end of Ventral tip of Posterior Adductor Muscle

Statistical analysis of shape and size

In the bivalves, the right and left halves are pairs of separated structures (Savriama and Klingenberg, 2011); the analysis of fluctuation asymmetry (FA) and right-left asymmetry was based on the matching symmetry of the test organism. Therefore, to study left-right asymmetry, the reflection was removed by transforming all configurations from one body side to their mirror images (Klingenberg and Mc-Intyre, 1998). A total of three independent group analyses were performed to describe the size and shape variations, 1) the right halves population; 2) the left halves population; 3) the right-left asymmetry analysis. Following a generalized procrustes analysis (GPA), a procrustes analysis of variance (ANOVA) was used to estimate the size and shape characteristics of three groups (right, left halves and right-left). Principal component analysis (PCA) was used to detect populations' shape variations (Rohlf and Slice, 1990; Jolliffe, 2002; Uba et al., 2019).

Multivariate regression analysis was used to analyze the size-related shape changes (allometry) of right and left shell halves. In this case, the size was fixed as an independent variable and the shape as a dependent variable and using 10000 permutation analyses for testing the significance level (Monteiro, 1999; Klingenberg, 2016; Zikic et al., 2017). Discriminant function analysis (DFA) was used to visualize the asymmetry of right and left halves (group fixed as *a priori*) (Villemant et al., 2007). Size and shape

similarities and dissimilarities between right and left halves were validated using 2B-PLS (two block-partial least square) analysis, with block one represented as size, and block two as shape (Rohlf and Corti, 2000). For developmental covariation analysis, we divided the shell into two blocks (dorsal and ventral regions), block 1 covers the umbo and cardinal tooth regions (Landmarks in block 2: 1, 2, 3, 4, 5, 6, 7, 9) and block 2 covered the shell shape regions (inner side of shell regions) (Landmarks in block 2: 8, 10, 11, 12, 13, 14, 15, 16). The covariation degree between two blocks was estimated using PLS1 within a configuration analysis with 10000 permutational analyses. The RV value below 0.5 is indicated as the covariation between the two blocks is low and above 0.5 is indicated as vice versa (Klingenberg, 2009). The entire statistical analysis was performed in MorphoJ v. 1.07a.

3. RESULTS

Size and shape variations within the population: Asymmetry

Within the right and left halves of the *V. cyprinoides* population do not exhibit significant shape and size variations. While comparing the left-right halves, it was proved that right and left shell asymmetry existed in the *V. cyprinoides.* Marginally insignificant ($p = 0.052$) size variation was observed in the asymmetry analysis, but they clearly show significant shape ($p = 0.008$) differences. Fluctuation asymmetry (FA) and directional asymmetry (DA) are only present in the right-left shape asymmetry analysis (Table 2).

Table 2 Population structure of *V. cyprinoides* from Koottayi estuary. Significant ($p<0.05$) result in side considered as the directional asymmetry (DA) whereas Side * Ind considered as the fluctuating asymmetry (FA)

Shell side	Analysis	Effect	SS	MS	df	F	P
Right	Centroid size	Individual	1187.941333	11.879413	100	1.00	0.4995
	Shape	Individual	0.23950893	0.000855389	2800	0.38	0.5405
Left	Centroid size	Individual	1126.308144	10.526244	107	0.91	0.8549
	Shape	Individual	0.28777594	0.0000960534	2996	0.47	0.5842
Right * Left	Centroid size	Individual	1143.315967	12.034905	95	1.05	0.0525
		Side	23.742919	23.742919	1	2.47	0.1197
		Side * Ind	914.818252	9.629666	95	1.16	0.2742
	Shape	Individual	0.237333044	0.0000892220	2660	1.11	0.0086
		Side	0.17205201	0.0061447145	28	70.15	<0.0001
		Side * Ind	0.23300357	0.0000875953	2660	1.09	0.0288

In the discriminant function analysis (DFA), we again proved that the right and left halves are entirely separated units and do not exhibit identical shape and size patterns (Figure 3A). Using multivariate regression analysis, we demonstrated that, significant ($p<0.001$) size-related shape changes (allometry) have existed in both the right and left halves, but they only cover a small percentage of allometric residues. The right halves covered the 4.7945% allometric residue and the left halves covered the 3.2131% of allometric residues. In terms of size and shape similarity between the right and left halve (2B-PLS analysis) of *V. cyprinoides,* marginally significant size overlap (Block 1) was observed between the right and left halves (Figure 3B) and opposite findings were observed in the shape (Block 2) differences between the right and left halves (Figure 3C). From the overall results, we confirmed that *V. cyprinoides* right and left halves are not identical and showed significant ($p<0.0001$) size and shape differences.

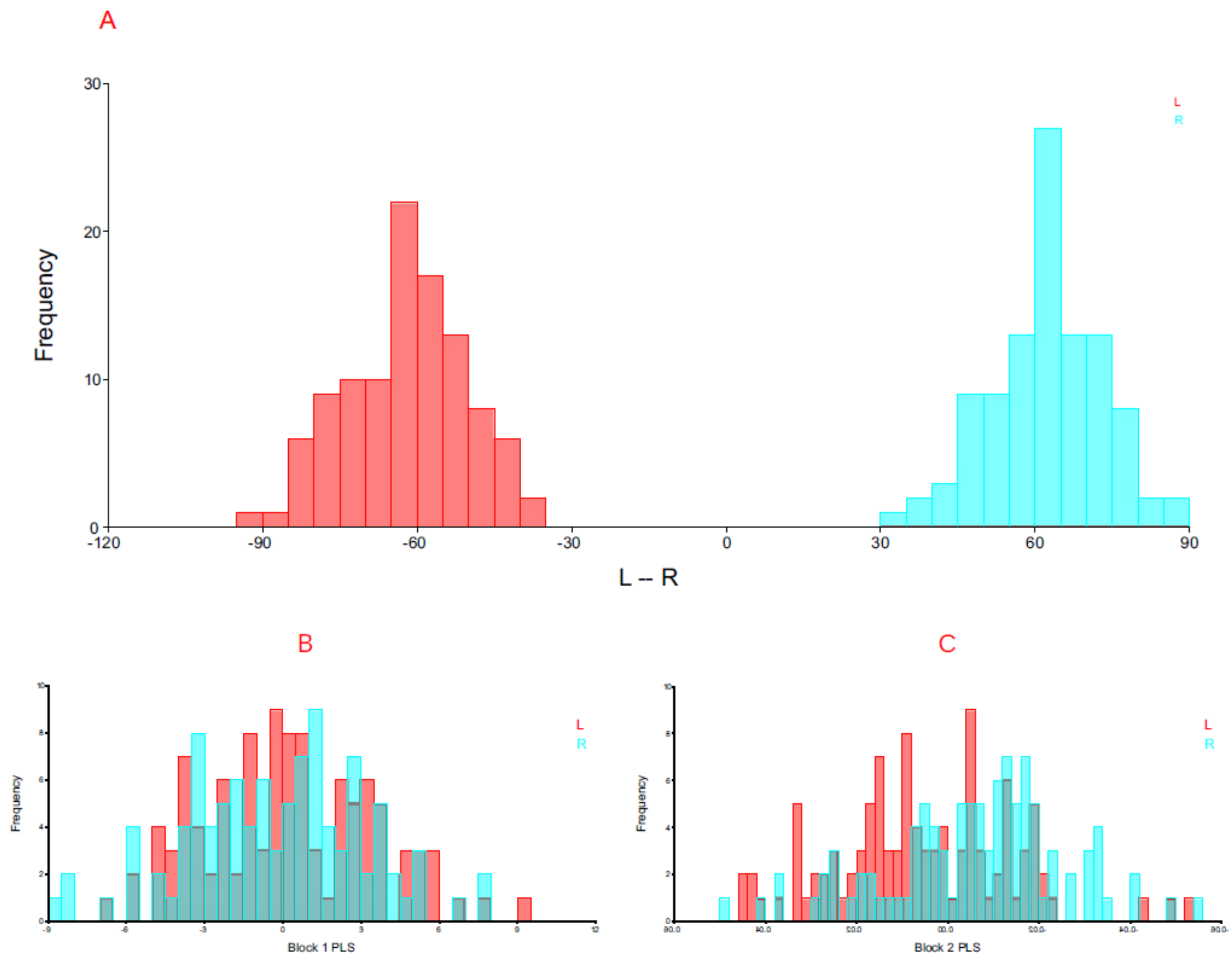

Figure 3 (A) Discriminant function analysis of right and left halves of endemic black clam; (B) Block 1 PLS size similarity and dissimilarity between right and left halves of endemic black clam; (C) Block 2 PLS shape similarity and dissimilarity between right and left halves of endemic black clam

Morphological variations of right and left shell halves

Principal component analysis (PCA) was used for quantifying the shape and size variations within the populations. We separately documented the morphological variations of the right and left halves. In the right halves, among the 28 PCs (Figure 4 A1), the first three PCs covered the 55.935% of variance (PC1 = 23.848%, PC2 = 19.766 & PC3 = 12.321%). In PC1 (Figure 4 A2), except for landmarks 6-11 & 14, all other landmarks displayed a high degree of deviation from the average point. The deviation of landmarks 12 and 15 and also 13 and 16 leads to the expansion of anterior and posterior adductor muscle scar areas. Same as in PC1 (Figure 4 A3), PC2 all landmarks showed deviation from the average position, the hinge region and umbo region landmarks pointed inward movements and also the outward movement of landmarks from the shell shape region alters the size and shape of the shell. In PC3 (Figure 4 A4), hinge region landmarks are highly conserved, they do not show substantial deviations from the average position. But the landmarks no. 10 and 8 showed too many deviations compared to other landmarks. It is indicated as the expansion of the shell in the dorsal region.

Among the 28 PCs in the left halves, the first three PCs covered 53.756% of the variance (Figure 4 B1). PC1 (Figure 4 B2) accounts the 27.210% of the variance, except for landmarks no. 8, 9 and 11, all other landmarks exhibit too many deviations from the mean shape. The same as in the right halves of PC1, the landmarks 12 and 15 (anterior adductor muscle) and 13 and 16 (posterior adductor muscle) showed too many deviations. The umbo region and cardinal tooth region landmarks moved into the inner side of shell regions. The counteract results were observed in PC2 (Figure 4 B3) and it covered the 15.850% of the variance, all landmarks were unevenly distributed in all directions, except for landmarks 7 and 10 and all other landmarks showed too many deviations from the mean position. In PC3 (10.696% of variance) (Figure 4 B4), except for umbo and cardinal tooth regions', all other regions landmarks are unevenly distributed in different directions.

From the overall individual population analysis (right and left halves), in the asymmetry analysis of right-left shell halves, each halve is distributed in a unique morphospace without any overlap. In the PCs morphospace distribution, the first two PCs covered 47.508% of population variance, PC1 accounted for 29.811% variance and PC2 is 17.697 % of the variance (Figure 5).

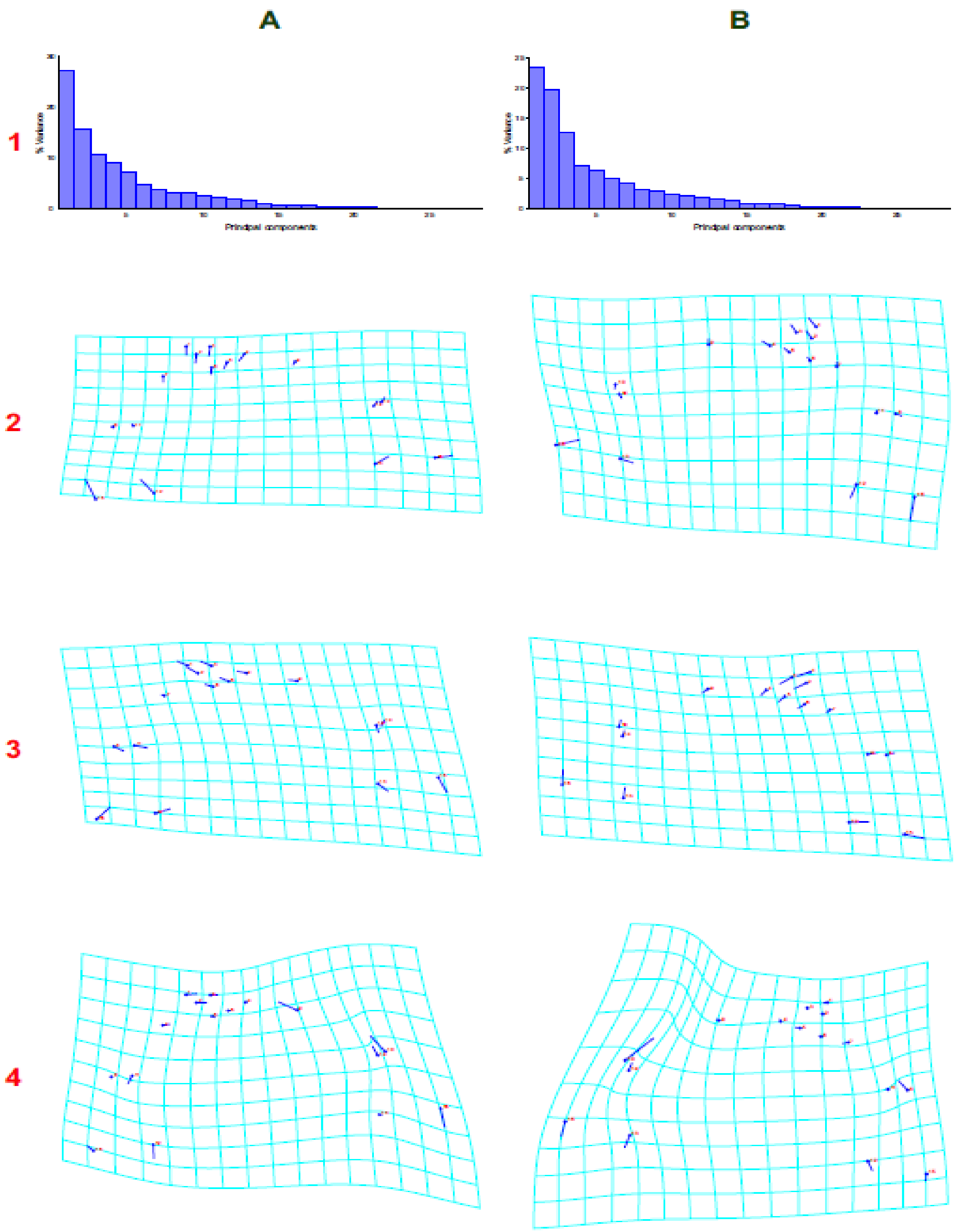

Figure 4 Principal component analysis of right and left shell halves of endemic black clam. (A1) % of variance of right shell; (B1) % of variance of left shell; A2-A4 - PC1, PC2 & PC3 of right shell; B2-B4 – PC, PC2 & PC3 of left shell

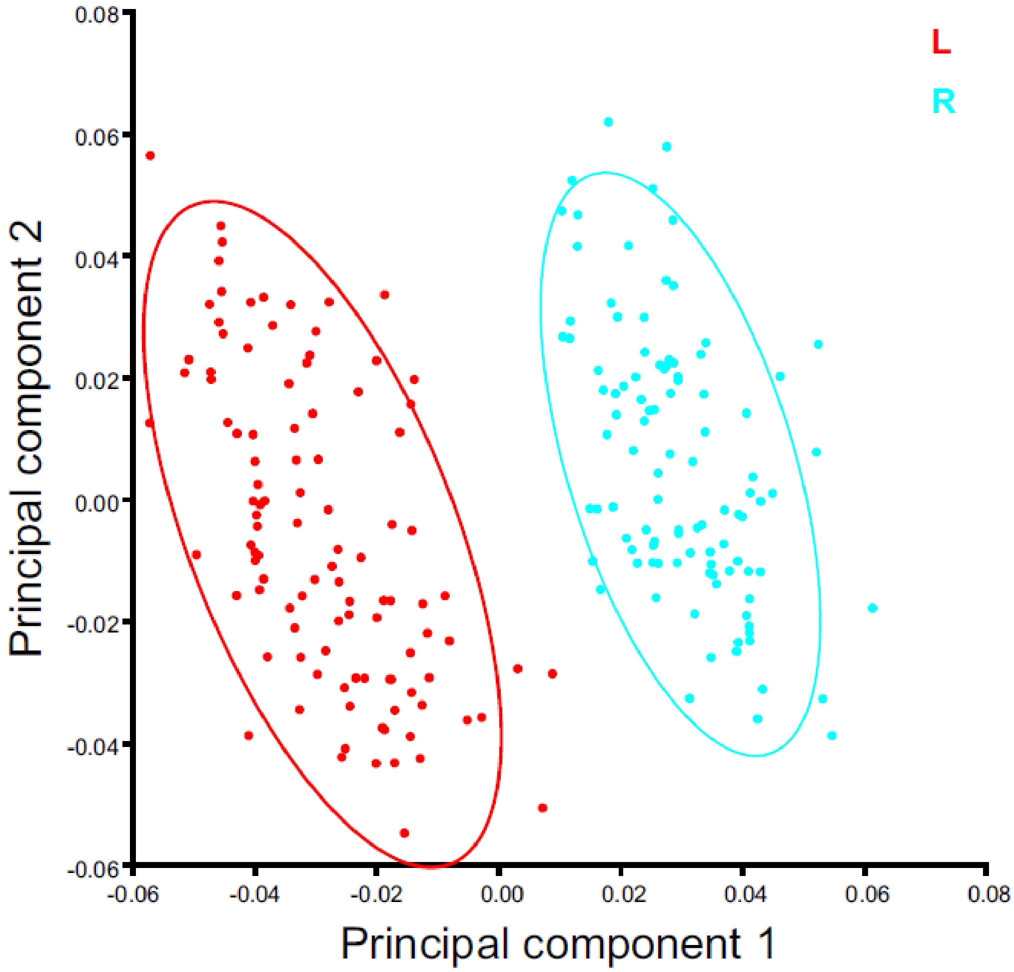

Figure 5 Morphospace shape and size distribution of right and left shell of endemic black clam

Developmental covariation analysis of *V. cyprinoides*

In both the right and left halves, the RV value is above 0.5; it indicated that covariation between blocks is strong. In the right halves (RV = 0.536, p<0.001) (Figure 6 A1, A2), block 1 landmarks showed a high degree of integration with the dorsal tip of the anterior adductor muscle (landmark no.11), the posterior end of the anterior lateral tooth (landmark no. 8), the ventral tip of anterior adductor muscle (landmark no.12) and the opposite end of the ventral tip of anterior adductor muscle (landmark no. 15). The landmark no. 10, 13, 14 and 16 showed less integration with other regions. In the left halves (RV = 0.567, p<0.001) (Figure 6 B1, B2), the same as in the right halves, a landmark within the block 1 displayed a high degree of covariation compared to other landmarks.

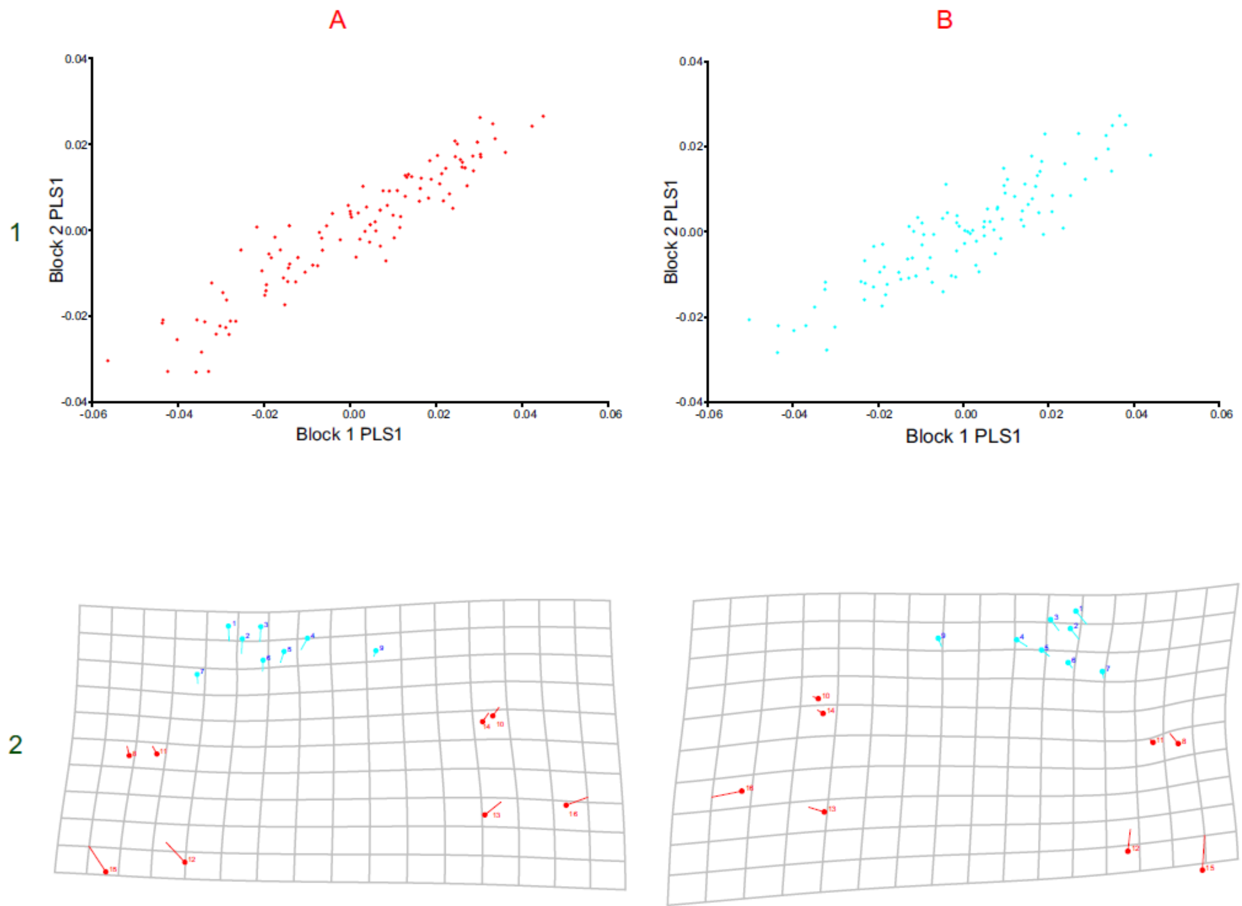

Figure 6 Developmental covariation between dorsal and ventral region of endemic black clam. (A1) % of covariation of right shell; (B1) % of covariation of left shell; (A2) Covariation between dorsal and ventral region of right shell; (B2) Covariation between dorsal and ventral region of right shell

4. DISCUSSION

Each species has its unique phenotypic variations, often related to its habitat (natural conditions – biotic and abiotic factors). During natural selection pressure, the species is forcefully to produce distinct forms (shape and size – morphological diversity) directly associated with their success and survival. Studying the morphological, phenotypic variations of local forms, would help to understand the origin of a new species (Hendry et al., 2007; Rasanen and Hendry, 2008; Dowle et al., 2015; Anand and Vardhanan, 2020). So, studying the local population phenotypic variations reveals the adaptation and stress response of an organism. Here, we describe the morphological variations of right and left shell halves of endemic black clam, *V. cyprinoides* population from Koottayi estuary, Kerala, south India.

Shell morphology is regulated by a set of heritable characters that can be shaped by environmental factors. Using GMM, we can study the shell variations into two separate components: Shape and size (Quenu et al., 2020). The shell's right and left halves did not show a significant difference in shape and size within the population. However, when considering the right and left shell asymmetry, marginally insignificant size and significant shape differences were observed. The existence of a significant shape-related FA and DA is the critical factor behind the right-left shell asymmetry. This asymmetry was suggested as the developmental instability of the local population (Koottayi estuary) of *V. cyprinoides.* Because, the FA is one of the most widely used tools for assessing developmental instability, both genotype and climatic factors regulate the developmental flux of the organisms, as evidenced by different genotypes exhibiting different levels of stability under the similar environmental conditions; and identical genotypes showing different levels of stability under varying environments (Waddington, 1942; Zakharov, 1989). Moreover, DA is developmentally regulated and likely correlated with adaptive significance, whereas FA is not associated with adaptation (Palmer, 1994). A possible implication of higher FA and developmental instability is that it can cause divergent phenotypes to express cryptic diversity (Gilbert, 2006). These phenotypes (expression of cryptic characters) are not the inheritance of acquired characters, but

rather a representation of an individual's existing genetic diversity that was previously impacted by the course of the developmental stability process (Nishizaki et al., 2015; Karthika et al., 2021; Anand et al., 2022). According to Woods, (2014), interactions between environmental factors and developmental noise can contribute to a higher range of phenotypes. This diversity arises from the stochastic processes of cell division and development, not from plasticity or adaptation. The fundamental mechanisms that induce the minor changes in FA-related morphology remain unclear. The stochastic switching of gene expression is the driving mechanism behind an organism's developmental instability (Klingenberg, 2003).

The black clam attains sexual maturity at a length of 11 to 15 mm (0.4 to 0.6 in). It does not show sex reversal or hermaphroditism. It is spawned twice a year, from May to August and from January to late March. The most important factor that causes spawning is the change in salinity, though the temperature is not a factor. The optimum spawning salinity is around 10-12 ppt (Arun, 2009; Ravindran et al., 2006). According to the National Green Tribunal (NGT), Kerala pollution control board, Govt. of India, Southern Zone (2016), reported that the Tirur-Ponnani River and Koottayi estuary were confronted with very serious environmental culmination, such as dumping of garbage, solid waste materials, oil spills, biomedical waste, etc. Apart from this, the Koottayi estuary is near the Ponnani harbor; the high level of anthropogenic influences has also changed the natural condition of the Koottayi estuary. Irregular water current from the Arabian Sea, high levels of pollution and human interference alter the native biodiversity of the Koottayi estuary (Sreenisha and Paul, 2016). These factors change the salinity, temperature and biodiversity of the estuary; it will ultimately lead to the developmental noise of organisms living in the estuary. We concluded that the high level of pollution and human intrusions is the crucial reason behind the FA of the black clam population of the Koottayi estuary. There is little information available on the functional aspects of FA in bivalves. Asymmetry in bivalve shells is known to cause drag (Olivera and Wood, 1997) and many thus increase susceptibility to wave action. Shell asymmetry can also increase desiccation, which is known to increase with valve gaps (Kennedy, 1976). Mismatches in valve size can make mussels more vulnerable to decapod predators crushing techniques (Elner, 1978). Further research is currently required to determine the functional implications of shell asymmetry.

From the multivariate regression analysis, significant allometry has existed in both the right and left halves of *V. cyprinoides*¸ even though it covers a low percentage of allometric residues. Our findings corroborate the previous results of Suja and Mohamed, (2012), according to this author, the biometric documentation of *V. cyprinoides* perfectly expressed the length-width and length-height relationships (positive allometric) even though they are collected from different stations of Vembanad Lake, Kerala, India. The finding implies that the growth in width and height is superior to an increase in length. This kind of positive allometry has been observed in some other bivalves, such as *Perna viridis* (Narasimham, 1981), *Donax Semistriatus, Spisula solida, S. subtruncata* and *Ensis siliqua* (Gaspar et al., 2002). The shell morphology variation analysis based on PCA revealed that each PC of the right and left shell exhibits different grades of variations, suggesting that a morphologically clear-cut differentiation existed in both the right and left halves of black clam. And these morphometric shape variations could be used for distinguishing the right and left halves of a black clam. In general, changes in the relative proportion of bivalve shells during growth are related to maintaining a suitable physiological ratio concerning environmental conditions (Rhoads and Pannella, 1970). Bivalve shells become progressively higher and wider during ontogeny to counter involuntary dislodgement, turbulence and currents (Hinch and Bailey, 1988). Shell morphology and the selective proportions of many bivalves are known to affect several environmental factors, such as latitude (Beukema and Meeham, 1985), depth and type of bottom (Claxon et al., 1998), tidal level (Dame, 1972), sediment type (Newell and Hidu, 1982) and burrowing behaviour (Seed, 1980).

Between primary and higher trophic levels species, benthic organisms constitute a critically important food chain. They change the bottom ecosystem structurally and maintain the biogeochemical cycling of nutrients and other substances. Because of their restricted mobility, benthic invertebrates are vulnerable to pollution and other environmental disturbances. In corollary, they were used as indicators for ecological health in general and focus on a wide range of environmental impact assessments (Sushama, 2014). Unraveling shell shape and size variations in a local environment reflect the natural selection pressure at work rather than random genetic drift (Giokas et al., 2014). From the 2B-PLS covariation study, we can confirm that the shape variable is more affected than size. And marginally significant covariation (dorsal and ventral region of the shell) was observed only in the PLS1 analysis. The covariation analysis (2B-PLS and PLS1) proved that shape-related developmental instability existed in *V. cyprinoides.* We previously stated that the Koottayi estuary is confronted with a high level of pollution and human interferences, altering the embryonic and post-embryonic development of black clam.

5. CONCLUSION

From the overall findings, two key conclusions can be drawn to explain the shell shape and size variations of *V. cyprinoides* from the Koottayi estuary: (1) this phenotypic variation can be the starting point for the expression of the cryptic diversity of black clam associated with their natural selection pressure; and (2) shell usually function as a highly integrated unit, reduction of covariation between the anterior and posterior shell regions; and high level of alteration of size and shape may lead to the extinction species from the Koottayi estuary. More genomic, reproductive, developmental and population studies are required to address our work's main findings. As a future recommendation, more sampling from other local populations across southern India is required to establish the evolution of natural selection and also to help to classify the native population. The analysis of an endemic organism's size, shape and asymmetry can effectively assess environmental health status. Our research will serve as a framework for future studies to determine the effect of pollution or environmental stress and human intrusion on endemic species and morphological variations can be used as a key tool for future studies.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Informed consent

Not applicable.

Ethical approval

The ethical guidelines are followed in the study for species observation & identification.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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